St4DeM

Manual v1.4

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Contents

Abbreviations

CBED	Converged Beam Electron Diffraction
CC	Cross-Correlation
DM	Digital Micrograph
DP	Diffraction Pattern
DI	Diffraction Image
EDS	Energy-Dispersive X-ray Spectroscopy
EELS	Electron Energy Loss Spectroscopy
EFTEM	Energy-Filtered TEM
EM	Electron Microscopy
FOV	Field of View
GIF	Gatan Imaging Filter
iDPC	integrated differential phase contrast
NP	Nanoparticle
ROI	Region of Interest
SI	Spectrum image
STEM	Scanning TEM
TEM	Transmission Electron Microscopy
UI	User Interface

1. Introduction

This software suite is for automatic data acquisition using the 4D-STEM method while operating a transmission electron microscope (TEM) in scanning mode (STEM). It acquires a converged beam electron diffraction (CBED) or diffraction pattern (DP) for every probe position within user defined region of interest (ROI) while scanning the beam across the sample, thus collecting a four-dimensional dataset. EFTEM mode and momentum resolved EELS are supported if a GIF camera is installed in the TEM. Additionally, dual beam EELS and EDS spectrum imaging can be used to acquire spectral data within the same ROI. External software AutoEM (https://doi.org/10.1007/s11051-019-4555-9) is incorporated within the suite, that allows the acquisition of multiple STEM images over a large area, automatic segmentation and 4D-STEM/EELS/EDS acquisition of unlimited particles. It also allows the acquisition of STEM tilt series for tomographic reconstruction coupled with 4D-STEM and EELS/EDS SI. In addition, some basic 4D visualization is provided including virtual imaging and integrated differential phase contrast (iDPC).

2. System Requirements and Installation

Gatan Digital Micrograph (DM), also known as Gatan Microscopy Suite (GMS) was chosen as the scripting environment for the St4DeM since it is available on a wide range of TEMs (Thermo Fischer, JEOL) with ready-made functions to control and automate the microscope and cameras. Therefore, DM must be installed on the TEM along with the *EMPlugin.dll* for TEM control and camera acquisition functions. Additionally, DigiScan hardware must be present for beam control.

St4DeM is provided under MIT license. If St4DeM has contributed to your research, please consider citing the article (Ref X.).

Installing St4DeM

Please note that this program is provided as it is, and the authors take no responsibility of any damage to the microscope hardware (over-exposure etc...) or data-loss. It is recommended that the user is constantly supervising the acquisition.

1.

Download the latest version St4DeM package from www.zenodo.com:
https://doi.org/10.5281/zenodo.7529702

Note that the St4DeM.dll is different for DM versions 2 and 3. Download the correct one depending also on the version of your DM.

2.

Open the folder "C:\Program Data\Gatan\Plugins". Note that "Program Data" folder is hidden by default. (Control Panel->File Explorer Options->View->Show hidden Files).
Extract the St4DeM.dll, St4DeM_x.x.gt1 into this folder. If the Microscope PC is different than the CCD camera PC, then repeat this step on the CCD PC. DM should be restarted. Additionally, extract the microscope control library FEI_functions.dll or JEOL_functions.dll to the same folder depending on your microscope vendor.

3.

If AutoEM options are of interest, extract the 'FijiAppND' folder directly to C: folder, such that the path is "C:/FijiAppND/". This is basically ImageJ software with AutoEM daemon script installed for image segmentation and microscope communication.

4.

St4DeM can be started by selecting the "St4DeM" menu command in DM. A dialog window user interface (UI) will appear as shown in Figure 1. This dialog consists of 8 different tabs: Main, Camera, Settings, AutoEM, q-EELS, Auxiliary, Analysis and Tomography.

Client/Server Setup

In the case of a TEM system consisting of two computers, a client-server configuration is needed. The CCD PC sends a message to the microscope PC, whenever a frame exposure has been completed, such that the beam re-positioning can take place. The basic communication between the client and server is done using a TCP connection. However, during acquisition, the frame ready -message is sent using UDP connection (ServerMode=1) or WinSock UDP connection (ServerMode=2), which are faster than TCP. Even faster RIO socket (ServerMode=3), is also incorporated but not tested.

One can only run the St4DeM acquisition command in the microscope PC, since the scanning control is currently not accessible through network. The microscope PC thus needs to be able to control the microscope and Digiscan. If there are cameras in other PCs to be used in the server mode, it is recommended to set the global tag "SU:AutoCreateServer" in that PC DM as true. One can also start the server by executing the following function as a script: "SU_ServerThread()", and delete it with "SU_DeleteServer()".

In Microscope PC:

1. Check the IPv4 address of the Microscope PC (e.g. 192.168.12.1)
2. Open the **global tags** in DM and set this value in
   (File -> Global Info -> Global Tags -> SU -> Server_IPAddress)
3. Check the IPv4 address of the CCD PC (e.g. "192.168.12.2"). Set this value in
   (File -> Global Info -> Global Tags -> SU -> Client_IPAddress)
4. Press File->Save Preferences.
5. Restart DM.

In CCD PC:

6. Repeat 1-3 in remote PC. The IPv4 address of the remote PC is then the "Server_IPAddress", and the microscope PC is the "Client_IPAddress".
7. (Optional) Define a port to be used. (default 1234)
   (File -> Global Info -> Global Tags -> SU -> Server_Port)
8. (Recommended) Set the global tag SU:AutoCreateServer as "true", at the remote CCD PC.
9. Press File->Save Preferences.
10. Restart DM.

AutoEM

Extract the Fiji.AppND folder directly to C: drive, such as the path is "C:\Fiji.AppND".

To run the AutoEM software within the St4DeM UI, ImageJ.exe should be running from the above path, and the Particle Sizer Daemon should be pressed (see next chapter).

3. Software Parameters

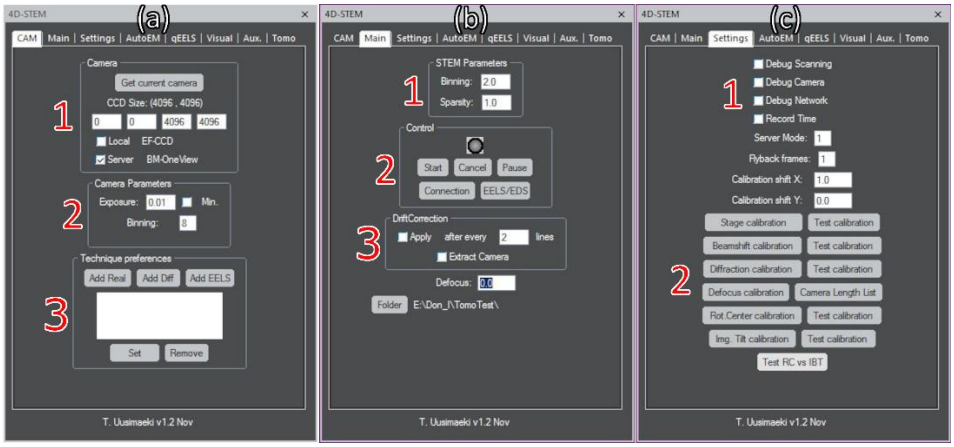

Figure 1. Three different tabs of the St4DeM user interface (UI) for basic 4D-STEM mode (DM3).

Camera Tab:

(1)

'Get Current Camera' –button will retrieve the name and CCD area of the current camera in use. Only current camera can be used, which can be changed using the menu command: "Camera-> choose camera" in DM (Make sure that Help -> User Mode -> Power User is selected). If the Server checkbox is on, then information about remote camera is retrieved. Press Connection button in the Main Tab to check if the TCP connection is working (should print the number 42). The four fields below the CCD area can be used to define what area of the CCD is used as [x_0, y_0, x_1, y_1], mainly used for non-square cameras.

(2)

Choose the camera exposure of a single frame image. If the Min. checkbox is chosen, minimum frame exposure of the camera is used. The binning field determines the binning of the CCD camera images.

(3)

The Technique Preferences box provides an easy auxiliary way to save and set camera length and diffraction shift values for different beam projection conditions (e.g. for changes in diffraction or imaging conditions). For AutoEM, these conditions should be set. Camera Length List calibration should be performed before using this feature.

Main Tab:

(1)

The binning field [float] in the STEM Parameters box determines the binning used within the ROI. For example, binning value of 2.0, takes 4 times less images and the pixel size will be 2 times bigger. Binning value of 1.2 takes images such that the pixel size of the 4D-STEM data stack navigation axis is px/1.2, where px is the original pixel size of the STEM image where the ROI is placed (e.g. in search or preview mode).

Additionally, the sparsity value between 0 and 1 allows sparse low dose scanning, which follows binomial statistics (Bernoulli distribution) to make it incoherent. E.g. sparsity of 0.5 only scans and acquires about half of the image pixels randomly.

(2)

The Control box is used to start, pause or cancel the 4D-STEM acquisition. The Connection button should print "42" if the TCP connection is working properly. The EELS/EDS button will acquire a 3D spectrum image using the ROI as a preference. The variables and settings of the EELS/EDS signal should be set on the DM spectrum imaging palette. In the Spectrum Imaging settings, disable subpixel scanning and auto-save. **In DM3, the spectrum imaging palette should be visible.**

(3)

For long experiments, automatic drift correction can be used. To apply it, press the Apply checkbox on the drift correction box within the Main Tab. Place a square annotation within the image, where the drift correction sub-image is acquired. Set the number of lines when the drift correction should take place. Before 4D-STEM acquisition, the software will acquire an image of the annotated area, and uses this for cross correlation to determine the stage drift, which is the corrected with beam shift. Hence, the beam shift calibration should be performed before using this feature. If the CCD camera blocks the STEM detector during scanning, press the Remove Camera checkbox such that the software removes the CCD camera before applying the drift correction.

The defocus value can be set for ptychography experiments. The defocus calibration should be done before using this feature. The Folder button sets the saving folder for AutoEM/Tomography data collection. All new AutoEM/Tomo experiments are saved under "Area_1" or "Tomo_1" folder respectively. If such a folder exists, then a new one "Area_2" is created etc.

The options for EFTEM, EELS and EDS will appear in the UI Main tab if they are supported by DM, which is automatically determined at start-up of the software.

EFTEM in diffraction studies is usually beneficial for removal of the in-elastically scattered electrons, which improves signal to noise ratio and contrast. The GIF camera has to be aligned with the chosen slit width before the 4D STEM acquisition. Choose the energy offset (usually 0 for zero loss filtering) and press the EFTEM check box for energy filtered 4D STEM acquisition.

Settings Tab:

(1) Do not use these for any actual data collection, since they will slow down the acquisition process.

The Debug scanning checkbox will print values into the result window related to the scanning process.

The Debug Camera checkbox will print values into the result window related to the Camera acquisition process and initialization.

The Debug Network checkbox will print values into the result window related to send and received TCP/UDP messages.

The Record time checkbox will result in an image at the end of the acquisition giving the measured frame exposure times. Some exposure times can be significantly larger than the mean one, this is related to the internal acquisition of dark reference images.

(2)

The Set flyback frame (default=1) is the number of left columns to add to count for flyback time of the beam. In DM3, these extra columns are deleted after acquisition, before displaying the 4D-STEM image.

The Server Mode (default=1) is the communication protocol during the acquisition between the microscope and CCD PC. UDP = 1 and Winsock UDP = 2.

Stage Calibration:

AutoEM software needs to calibrate the stage to correctly move the stage.

1. To start, set the stage X and Y coordinates to zero (less than 0.2) and press the "Stage calibration" button. This process will determine the mode of the stage shift.

2. Then with aligned microscope, good imaging conditions and a sample with some detailed contrast e.g. nanoparticles, press the "Stage calibration" button again. Two images "source" and "shifted" should appear with an error that a filter does not exist.

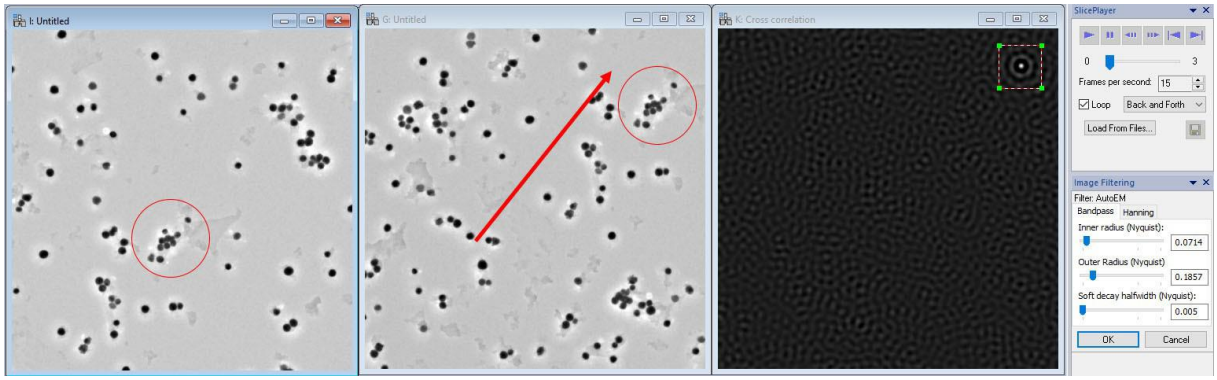

Figure 2. Filter parameter adjustment for Stage Calibration within DM2. Notice how the CC peak location and direction of the red arrow coincide. Always check that the shift and the CC peak location are in the same direction. If the shift is too large the CC peak might appear in the lower left position, giving erroneous results.

DM 3:

Go to Technique manager and press the Techniques button, then choose Process Image. Press the lower settings button in the Image Filtering palette and add a new filter. Choose "Combined Filter" and name it exactly "AutoEM" without the quotes. Press OK. Then choose the AutoEM filter and press Edit. On the left choose add the Hanning Window Filter by pressing the arrow to the right button. Do the same with Bandpass Filter. In the Image Filtering palette, choose the AutoEM filter and press the Live (2) button. Choose the "source" and "shifted" images. Set the Hanning window value to about 0.2 and choose an Inner Radius value of the Bandpass filter to make the cross-correlation (CC) peak as sharp as possible.

DM 2:

Press the "Manage Image Filters" button in the Image Filtering palette and add a new filter. Choose "Combined Filter" and name it exactly "AutoEM" without the quotes. Press OK. Then choose the AutoEM filter and press Edit. On the left choose Hanning Window Filter and press the arrow to the right button. Do the same with Bandpass Filter. In the Image Filtering palette, choose the AutoEM filter and press the "Live Setup (cross-correlation)" button. Choose the "source" and "shifted" images. Set the Hanning window value to about 0.2 and choose an Inner Radius value of the Bandpass filter to make the cross-correlation (CC) peak as sharp as possible.

With the CC filter optimized for the current sample, then press the Stage Calibration button again. Keep the Stage Calibration Y value zero and increase the X value until you observe the peak of the CC (Figure 2) to be as close as possible to the edge of the image (pass value > 0.8). Make sure that the peak is into the same direction as the shift of the stage. Then keep the X value zero, and increase the Y value similarly.

Beam Shift Calibration:

St4DeM software needs to calibrate the beam for correct beam shifts when using drift correction. This calibration is performed similarly as the stage calibration. Note that the needed X and Y values for the shift can be several orders of magnitudes larger than in stage calibration.

Diffraction Shift Calibration:

St4DeM software needs to calibrate the projection system for correct diffraction shifts when using q-EELS. This calibration uses the CCD camera and must be done on each camera separately. Place the focused probe stationary on a sample with good diffracting conditions. Keep the Stage Calibration Y value zero and increase the X value until you observe the peak of the CC to be as close as possible to the edge of the image (pass value > 0.8). The AutoEM filter settings may have to be changed for diffraction patterns. Make sure that the peak is into the same direction as the shift of the diffraction pattern. Then keep the X value zero, and increase the Y value similarly. Note that the needed X and Y values for the shift can be several orders of magnitudes smaller than in stage calibration.

Defocus Calibration:

The defocus calibration asks to press eucentric focus / STD button, and it will determine how much the DAC value in DM changes the defocus. Check that the defocus value actually changes to zero after pressing the eucentric focus /STD button.

The 'Rotation Center' and 'Image Tilt' buttons refer to precession diffraction calibrations, which are not yet in use.

Camera Length List:

This calibration creates a list of the camera lengths and their indexes and should be done before using the Technique Preferences box in the Camera Tab.

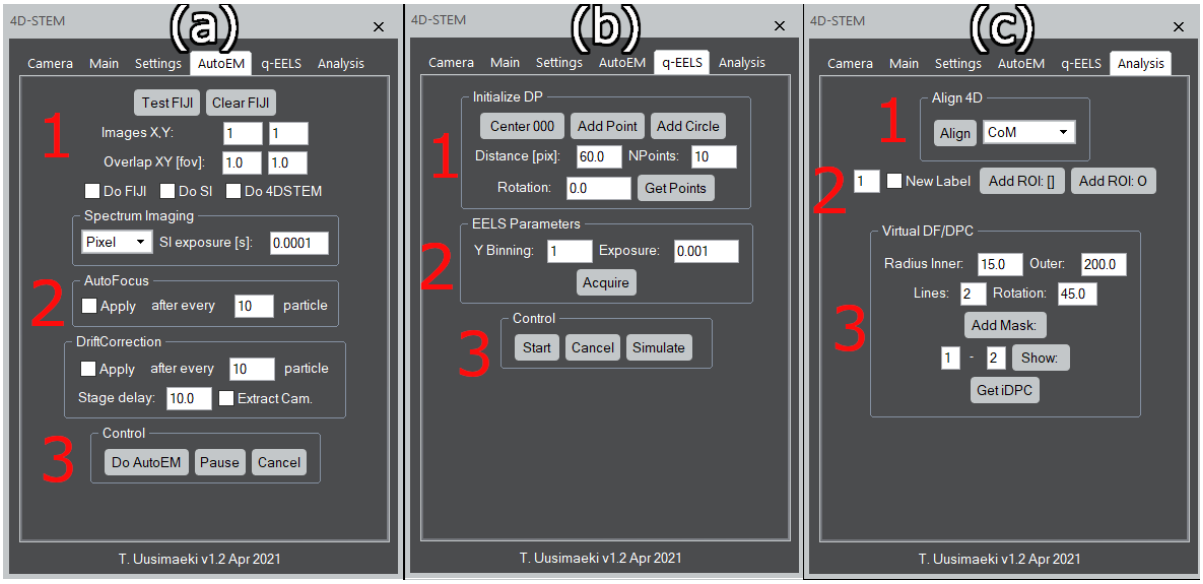

Figure 3. Three different tabs of the St4DeM user interface (UI) for advanced modes (DM3).

AutoEM Tab:

This tab incorporates an external software called AutoEM [2] into the St4DeM suite. AutoEM allows the acquisition of multiple images while shifting the stage to form a montage image of the sample. Each image can be segmented using the Particle Sizer [3], to calculate the particle size distribution of e.g. nanoparticles. Additionally each particle can be analyzed with 4D-STEM, EDS and EELS spectrum imaging.

Make sure that the Fiji software is running and press Plugins->NanoDefine->ParticleSizer Daemon…

(1)

To test the functionality of the segmentation process, acquire an image with same binning as the final images to be recorded. Press Test FIJI. A segmented image should appear. If the segmentation is not satisfactory, go to the settings of the Particle Sizer (Plugins->NanoDefine->Settings Manager) and adjust the segmentation parameters. The Settings Manager window should be closed for changes to take place. Information about the segmentation parameters can be found at [3].

In "Images X, Y" one can set the number of images to record in X and Y direction. The software will start the acquisition at the upper left position. The "Overlap XY" will determine if overlapping is required e.g. for stitching the images together later to form a mosaic image. A value of 0.8 will leave 20% overlap between the images in X and Y directions separately, and the value of 1.2 would shift the stage 1.2 * field of view to reassure that no overlap would take place.

Test the software by setting the Images X,Y to (1,1). A single acquired image with the segmentation should appear along with the particle size distribution and results image. The results image shows many calculated attributes of the segmented images:

Frame – Label – X – Y – Area – Area Conv. Hull – Peri. – Peri. Conv. Hull – Feret – Min. Feret – Maximum Inscriped Circle Diameter – Area equivalent Circle Diameter – Long Side Length MBR – Short Side Length MBR – Aspect Ratio – Area/Peri. – Circ. – Elong. – Convexity – Solidity – Num. of Holes – Thinnes Rt. – Contour Temp. – Orientation – Fract. Dim. – Fract. Dim. Goodness

For more information about the values in the results image, please refer to [3].

The radio buttons Do FIJI, Do SI and Do 4DSTEM, will enable the segmentation process for particle size distribution, EDS/EELS spectrum imaging and 4DSTEM acquisition respectively. The EDS/EELS acquisition parameters should be set in the DM spectrum imaging palette.

The SI exposure time is for EDS and/or EELS spectrum imaging. Note that no over exposure safety mechanisms exist within the St4DeM suite. One can set the exposure time per pixel or as a total duration of the spectrum imaging mapping.

(2)

The Autofocus procedure is an optimization routine based on [4]. Press the Apply checkbox to enable it and choose after how many particles it should be applied.

The Drift Correction works similarly as in Main Tab. Here an automatic routine is used to search for a region of interest with optimum contrast properties within the image - to be used for the CC procedure. The stage delay is related to how many seconds the program pauses after stage shift has been applied, and is not related to drift correction itself.

(3)

The Control box is used to start, pause or cancel the AutoEM acquisition.

q-EELS Tab:

This tab is used for momentum resolved EELS experiments (beta version).

(1)

The Initialize DP box provides some methods to test the EELS signal coming from different parts of the DP. The 'Center 000' -button searches for the direct beam and uses diffraction shift to move the pattern to the centre. Diffraction calibration should be performed before using this feature. One can place single points into the DP by pressing the Add Point button, and then moving them to appropriate location. If there is a line ROI within the DP, a series of points are added on both sides of the line according to the Distance parameter and Number of Points. If the distance is zero, points are added to the line directly. Additionally, a resizable circle can be added within the image with a given 'Number of Points' around it, and then 'Rotation of the Points' can be applied if needed. In all cases, 'Get Points' -button must be pressed to register the points before starting the acquisition.

(2)

The 'EELS Parameters' box acquires a 2D EELS spectra. The y binning determines (2-256) the horizontal binning of the spectra, the smaller the faster the acquisition.

(3)

The 'Control' box is used to start or cancel the q-EELS acquisition. The 'Simulate' button, applies the diffraction shift as determined by the registered points to check the validity of the Points. Use live view of the CCD camera before pressing this button.

Analysis/Visual Tab:

(1)

The 'Align 4D' box is used to shift all 000 peaks to the centre of the images. The 'Center of Mass (CoM)' -option works well with data acquired using high camera length (e.g. ptychography). And the auto-correlation option works better with data acquired using low camera length, having small well-defined points around the 000 peak. The 'Auto-Correlation' alignment computes an auto-correlation image, which is always centred, to which the image is then cross-correlated and aligned.

(2)

With the 4D-STEM image as front image, press the "Add ROI []" button. This will add one-pixel square ROI into the 4D image and opens a new 2D DP corresponding to that pixel. One can then resize and move the ROI within the 4D-STEM image, and the DP will automatically update. By setting the New Label checkbox un-selected, one can add new ROI, and the mean value of all ROIs is calculated to the DP. By checking the New Label checkbox, a new DP will appear for the new ROI with a corresponding label.

Similarly, by pressing the "Add ROI O" button while the DP image is selected as front image, a resizable and movable circular ROI is added to the DP and a new 2D image will appear, with the same size as the x and y components of the 4D-STEM image (navigation axes). The new 2D image is displaying the integrated intensity inside the circular ROI only, for e.g. dark/bright field imaging. This works only in DM3.

(3)

The 'Virtual DF/iDPC' box allows virtual bright/dark field images to be extracted from the 4D data (DF, HAADF). By dividing the virtual annular detector into segments, also integrated Differential Phase Contrast images can be obtained (iDPC). If the variable 'Lines' is zero, by pressing the 'Add Mask' -button will add an annular ROI with given 'Inner and Outer' diameters. This will open a new DF image with integrated intensity inside the annular virtual detector (ROI). If the variable 'Lines' is 1, a line ROI will be added to the annular ROI, dividing the virtual detector into 2 labelled segments. Using the 'Rotation' variable, the Line ROI can be rotated. By pressing the 'Show' button, a new image will appear, where the intensity is subtracted between the selected two segments. E.g. adding a line ROI with rotation zero, and then pressing 'Show', will give a vertical difference image of the segments. Putting 'Rotation' to 90 degrees, and pressing 'Show' button again, will give the horizontal difference image. Then pressing 'Get iDPC' -button and choosing these 2 images, will give a complex image showing the x and y directions of the difference, and an iDPC image. One can also extract the CoM (center of mass) images from the 4D-STEM data by pressing the 'Get CoMs' button. This will display the CoMx and CoMy images. Pressing the 'Get iDPC' -button and choosing these two images will also yield a DPC image. A fitted plane is automatically subtracted from the CoM images to remove the d-scanning effect.

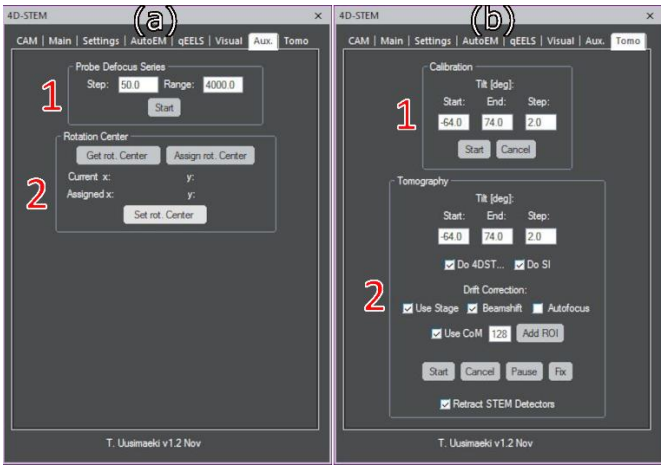

Figure 4. Auxiliary and tomography tabs of the St4DeM user interface (UI) for advanced modes (DM3).

Auxiliary Tab:

(1)

'Probe Defocus Series' box is an auxiliary method if a defocus series of the probe is needed for data analysis. It uses the CCD camera to take an image of the probe while changing the defocus value. The defocus is changed as set in the defocus range and step size and takes a number of (range/step + 1) images arranged as a 3D stack.

(2)

'Rotation Center' box is related to precession diffraction method, and is not yet in use.

Tomography Tab:

(1) This is still in beta version. The Tomography tab is not tested in JEOL microscopes, or DM version 2.

The 'Calibration' box allows to measure the stage drift during the tilt series after goniometer tilt changes. Go to the maximum minus tilt plus a bit further (e.g. if max tilt is - 64°, go to -64.4° because of backlash). Assign maximum plus tilt, then press Start. The 'Stage Drift', and 'Beam Shift' Calibration has to be done before using this feature, and the 'AutoEM' filter should be checked for the current sample. Currently, no interpolation is done between the tilt angles. So, do the calibration exactly the same, as you will do the actual tilt series (e.g. use the same +- tilts and tilt step size).

(2)

The 'Tomography' box allows for basic STEM tomography tilt series acquisition, if the 4D-STEM and EELS SI checkboxes are unchecked. If tilt calibrations are present, they will be automatically used after tilt changes. The delay after the tilt change can be set in the tags. If the 'Use Stage' checkbox is selected, an extra Stage drift check will be done using stage positioning. If the 'Use Beamshift' is selected, an extra Stage drift check will be done using beam shift (faster). If 'Autofocus' is selected, an automatic defocus check is done after every tilt change. Note that this will not work in STEM microbeam mode in Thermo Fisher microscopes since the TEM Scripting SDK does not allow to change the defocus, when it is controlled by the C3 lens. If 4D-STEM and/or EELS/EDS SI are selected, the software will acquire these data after the STEM image has been acquired. This requires a ROI to be put on the View image around the object of interest (one can use the 'Add ROI' button, with the value as the size of the ROI), before the acquisition is

started. The 'Use CoM' check box, centers the ROI around the object of interest before the 4D-STEM acquisition (after the Stage and Beamshift corrections).

The 'Fix' button can be used to pause the tilt series every time after the tilt change for manual corrections.

If the STEM detector blocks the DP signal going into the CCD, assign the 'Retract STEM detectors' checkbox. This will retract the STEM detectors before the 4D-STEM acquisition, and then re-insert them after. This feature requires that the 'STEM Detector Calibration' is done in the Calibration tab. Since it is not possible to retract or insert STEM detectors using the TEM Scripting Interface (to the authors knowledge…), this feature was accomplished using scripted control functions of the mouse pointer. Every time after a tilt change, when acquiring a 4D-STEM - the mouse pointer will be set onto the Tecnai User Interface 'Retract STEM detectors' button. Therefore, this button should be visible before starting the tilt series, and one should not touch the mouse before the 4D-STEM acquisition starts. There will be a 3 second alert window in DM every time this takes place…

4. Running the Software

To run the software, press St4DeM menu command in DM.

Quick setup:

- Define CCD area parameters, exposure time and CCD binning in the Camera Tab.
- Define STEM binning in the Main Tab.
- Take an image using the Digiscan Dialog Window (preview or capture).
- Define a ROI in the DS image around your object to be scan-imaged.
- (optional) Use DigiScan beam spot feature to place the beam stationary away from your ROI. Check with the camera, that the CBED image is fully covered by the CCD and there are no STEM detectors etc. blocking it. Use diffraction shift / PLA to position the CBED in the middle of the CCD. Stop the camera.
- (optional) If using server mode, press Connection button to verify TCP connection.
- Press Start button.

The scan-imaging progress should be seen as a percentage in the DM progress window, and a cross-ROI rastering within the ROI. If not, press Cancel and try to repeat the above procedure with debugging on.

5. License

The MIT License (MIT)

6. Troubleshooting

St4DeM:

Due to DMs internal mechanisms, placing the ROI into the view image can sometimes give errors. Please use at least preview quality images for placing the 4D-STEM ROI.

AutoEM:

Pressing the 'FIJI test' button sometimes gives errors in FIJI. Usually this happens if a too small ROI is used for selected FIJI parameters. Close the Fiji images and press 'Clear FIJI' button in DM. Then restart the AutoEM daemon in FIJI. Use whole images only if the problem persists.

Visualization:

Occasionally, the connection between the ROI and the DP is lost, and moving the ROI does not update the DP anymore. DM has to be re-started to fix this. Sometimes this can also crash DM! The search for this bug is ongoing…

7. References

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